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Cow's milk related gastrointestinal symptoms in adults

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ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine of the University of Helsinki, for public discussion in Lecture Hall 2, Haartman Institute, Haartmaninkatu 3
On August 7th 2009, at 12 noon

Helsinki 2009

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ISBN 978-952-92-5732-4 (pbk.)
ISBN 978-952-10-5622-2 (PDF)

Helsinki University Print
Helsinki 2009

Abstract

Gastrointestinal symptoms are frequent in the adult working-age population. Sufferers often relate them to ingested food substances, especially cow's milk. The purpose of this study was to evaluate subjective food-related gastrointestinal symptoms and their relation to cow's milk. This was done by determining the genotype of adult-type hypolactasia, measuring antibodies against milk protein, and screening the most common cause for secondary hypolactasia, namely coeliac disease, in adults in primary care. The aim was also to evaluate the clinical role of the laboratory tests used in the study.

The whole study group comprised 1900 adults who gave a blood sample for the study when they attended a health care centre laboratory for various reasons. Of these 1885 completed a questionnaire on food-related gastrointestinal symptoms. The response rate was 99%, which is exceptionally high.

Study No. I evaluated the prevalence of adult-type hypolactasia and its correlation to self-reported milk induced gastrointestinal symptoms. The questionnaires requested data on dairy consumption and abdominal symptoms. The testing for hypolactasia was done by determination of the C/T_{-13910} genotypes of the study subjects. The results show that patients with the C/C_{-13910} genotype associated with adult type hypolactasia consume less milk than those with C/T-13910 and T/T-13910 genotypes (high lactase activity).

Study No. II evaluated the prevalence and clinical characteristics of undiagnosed coeliac disease in the whole study population with transglutaminase and endomysium antibodies and their correlation with gastrointestinal symptoms. The prevalence of undiagnosed coeliac disease was 2%, which is surprisingly high. Serum transglutaminase and endomysium antibodies are valuable tools for recognising an undiagnosed coeliac disease and should be used in outpatient clinics for diagnosing gastrointestinal symptoms.

In the study No. III the evaluation of milk protein IgE related hypersensitivity was carried out by stratifying all 756 study subjects with milk related problems and randomly choosing 100 age and sex matched controls with no such symptoms from the rest of the original study group.

In the study No. IV 400 serum samples were randomly selected for analyzing milk protein related IgA and IgG antibodies and their correlation to milk related GI-symptoms.

The measurement of milk protein IgA, IgE or IgG (studies No. III and IV) did not correlate clearly to milk induced symptoms and gave no clinically significant information; hence their measurement is not encouraged in outpatient clinics.

In conclusion, this large study of milk-related gastrointestinal symptoms in adults shows that GI-symptoms are subjectively very common. Adult type hypolactasia is often considered the reason for them, and determination of the C/T_{-13910} genotypes is a practical way of diagnosing the possibility of adult type hypolactasia in an outpatient setting. Undiagnosed coeliac disease, the possible cause of secondary hypolactasia, should be actively screened and diagnosed in order to apply a gluten free diet and avoid the GI-symptoms and nutritional deficiencies. Cow's milk hypersensitivity in the adult population is difficult to diagnose since the mechanism in which it is mediated is still unclear.

Measuring of cow's milk protein specific antibodies IgE, IgA or IgG do not correlate with subjective milk-related GI-symptoms.

Acknowledgements

This study was carried out at the Hospital for Children and Adults, the Hospital for Skin and Allergy diseases and at the Department of Medical Genetics, University of Helsinki.

Firstly, I would like to express my deepest gratitude to my supervisor Docent Kaija-Leena Kolho who has been admirably patient and overwhelmingly helpful in introducing the scientific world to me. Her encouragement and support have helped me to achieve my goal. She guided me through the fields of coeliac disease and cow`s milk allergy, and acted as a supervisor and "gun-powder" during the entire study.

I am deeply thankful to Docent Irma Järvelä who shared her wide knowledge of adult type hypolactasia with me and was the person to initiate the study together with Docent Kaija-Leena Kolho. She played a crucial part in the first and largest part of the study: the study of the prevalence of adult-type hypolactasia and its correlation to self-reported milk induced gastrointestinal symptoms.

I am grateful to Professor Erkki Savilahti, who helped in the milk-protein IgG and IgA section of the study by taking part in the article writing as well as organizing the laboratory work. Professor Tari Haahtela and Ph.D. Peter Elg from the Skin and Allergy Hospital are thanked for their assistance in organizing the laboratory work in the milk protein IgE section and providing premises for the milk challenge test.

Antti Kotamies is thanked for his excellent work on statistics and Ph.D. Heli Rasinperä for her co-work in the section on adult-type hypolactasia. Hanna Komu is thanked for her assistance in analyzing the gene-tests for adult type hypolactasia as well as helping in organizing the collected data in Excel format. Professor Hilpi Rautelin is thanked for analyzing the serum samples for *Helicobacter pylori*.

I would also like to express my gratitude to all the personnel of the Espoo City Health Care Centre and Tilkka Military Hospital laboratories who took the serum samples and collected the questionnaires. Their work was crucial in achieving the exceptionally good results. The personnel in the laboratory of the Department of Medical Genetics are thanked for their prompt and accurate work on determining the C/T-$_{13910}$ genotypes

Professor Markku Heikinheimo is thanked for being in the support and advisory group for this research.

All co-writers of the articles who are not named above are thanked for their support.

My family is thanked for their understanding of the time spent on the study. My husband for the weekends spent at home due to the reading and writing, and my children accepting the answer:"I am busy writing."

Contents

List of original publications

This thesis is based on the following publications:

I Anthoni SR, Rasinperä HA, Kotamies AJ, Komu HA, Pihlajamäki HK, Kolho KL, Järvelä IE. Molecularly defined adult-type hypolactasia among working age people with reference to milk consumption and gastrointestinal symptoms. World J Gastroenterol. 2007 Feb 28;13(8):1230-1235

II Tikkakoski S, Savilahti E, Kolho KL. Undiagnosed celiac disease and nutritional deficiencies in adults screened in primary health care. Scand J Gastroenterol. 2007;42(1):60-65

III Anthoni S, Elg P, Haahtela T, Kolho KL. Should milk-specific IgE antibodies be measured in adults in primary care? Scand J Prim Health Care. 2008;26:197-202

IV Anthoni S, Savilahti E, Rautelin H, Kolho K-L. Milk protein IgG and IgA: the correlation with milk induced gastrointestinal symptoms in adults, accepted; in press World J Gastroenterol.

The first publication "Molecularly defined adult-type hypolactasia among working age people with reference to milk consumption and gastrointestinal symptoms" has also been used in the thesis of Heli Rasinperä.

The publications are referred to in the text by their roman numerals.

Abbreviations

ARA	Reticulin antibody
AGA	Gliadin antibody
BHT	Breath hydrogen test
BMI	Body mass index
CD	Coeliac disease
CMA	Cow`s milk allergy
DGP	Deamidated gliadin peptide
DNA	Deoxyribonucleic acid
EmA	Endomysium antibody of IgA class
GI	Gastrointestinal
HLA	Human Leukocyte Antigen
IBS	Irritable bowel syndrome
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IU	International unit
LTT	Lactose tolerance test
C/C	Cytosine/Cytosine
C/T	Cytosine/Thymidine
PCR	Polymerase chain reaction
RAST	Radioallergosorbent test
S-TfR	Serum transferrin receptor
T/T	Thymidine/Thymidine
tTG-ab	Tissue transglutaminase antibody
TG2A	Transglutaminase antibody of IgA class

1 Introduction

According to clinical experience in outpatient clinics, it is common for attenders to suspect various food-related reasons as being causative of their gastrointestinal symptoms. It is often a challenge for a physician to diagnose the origin of these symptoms. The symptoms do not normally cause absence from work, but can reduce working capacity by causing inconvenience and lack of concentration.

Cow's milk, and especially lactose, is more often considered to be the reason for gastrointestinal symptoms than true lactose malabsorption (Caroccio et al 1998; Vesa et al 2000). This is very important since milk consumption in Finland per capita is high; in 2007, this was 3.76 dl/ person/ day, and if we include yoghurt and sour milk-products the consumption was 5.05 dl/person/day. Ice-cream consumption was 0.36 dl/person/day (www.maitojaterveys.fi). Hence the true correlation of GI-symptoms with ingested milk is most meaningful.

The bases of this study were to test the newly implemented C/T_{-13910} genotype–determination in clinical practice in the adult population; its correlation to milk induced gastrointestinal problems: and its applicability for testing of adult-type hypolactasia in primary care.The aim was also to clarify possible confounding factors such as allergy to milk protein, and coeliac disease, which is the major cause of secondary hypolactasia.

This study is the first large-scale study performed among the working age adult population in this area of Finland, and the perspective is clinically orientated.

A thorough questionnaire on food- and especially milk-related GI-problems was generated and pretested. Four healthcare centres in Espoo city and the outpatient clinic of the Military Hospital Tilkka agreed to take part in the study. The approval of the Ethical Committee was granted in December 2003 and the study started in February 2004. In a three-month period, 1900 volunteers gave a blood sample for the study when visiting the outpatient laboratory for various reasons. Of these, 99% completed the questionnaire.

The questionnaires were analyzed and four different types of research approaches were generated:

1. C/C_{-13910} genotype-testing related to self-experienced symptoms of hypolactasia
2. Undiagnosed coeliac disease; its prevalence and correlation to clinical symptoms
3. The role of cow's milk protein IgE in milk hypersensitivity in adults
4. The role of cow's milk protein IgG and IgA in milk hypersensitivity in adults.

All serum samples were also tested for *Helicobacter pylori* antibodies, and the possible role of *Helicobacter pylori* infection in nonspecific gastrointestinal symptoms resembling those induced by primary hypolactasia or milk protein allergy was evaluated.

2 Review of the literature

2.1 Adult-type hypolactasia

2.1.1 History and clinical manifestations

In primary adult-type hypolactasia, lactase activity is high at birth and gradually decreases during childhood and adolescence. Primary hypolactasia is also called lactase nonpersistence and is a normal physiologic phenomenon among mammals. For newborns, milk is a valuable nutritional source, but its importance diminishes after weaning. There are also other types of lactase deficiencies and the terminology is clarified in Table 1 below.

Table 1 *Terminology of lactase deficiencies.*

Lactose intolerance	symptoms from lactose
Hypolactasia	very low lactase activity
Alactasia or lactase deficiency	total lack of lactase activity
Lactose malabsorption or lactose maldigestion	poor lactose hydrolysing capacity
Primary lactose malabsorption or lactase non-persistence or adult-type hypolactasia	manifestation during development, irreversible
Secondary lactose malabsorption	due to mucosal injury, reversible
Congenital lactase deficiency	manifestation immediately after birth, rare congenital disease

Gene mutation of high lactase activity is thought to coincide with the development of dairying within the last ten thousand years, hence being a relatively new evolutional development and explaining the higher prevalence of lactase persistence in Northern and Central Europe, America and Australia (Flatz et al 1987). In Finland the prevalence of primary lactose maldigestion is 17%, whereas in Central Asia it is 90-100% (Sahi 1994). Ethnicity seems to have an influence in which age lactase activity degrades: in the Thai population, with almost 100% having adult-type hypolactasia, the average age of losing lactase activity is two years, and in Finland during school age or even later (Kolho et al

2000; Rasinperä et al 2005; Seppo et al 2008). The factors responsible for this activity degradation are not yet known. The lactase activity is not sustained even when the milk consumption would be continued (Schrimshaw et al 1988).

Lactase, or more precisely lactase-phlorizin hydrolase, is needed for hydrolyzing milk sugar lactose to galactose and glucose in the brush-border membrane of the small intestinal epithelium, after which they can be absorbed. Without lactase-phlorizin, hydrolase lactose cannot be absorbed and causes symptoms such as bloating, flatulence and diarrhoea in the majority of subjects. There are, however, considerable individual variations in the symptoms due to differences of microbial flora of the colon and sensitivity to stretching of the mucosal membrane, as well as to the amount of lactose consumed (Vesa et al 1998; Egert et al 2006).

Subjects with low lactase activity and gastrointestinal symptoms often have a better quality of life if they reduce their milk consumption or use delact milk. But milk is often unjustifiedly considered the reason for the GI-symptoms and self-diagnoses of "milk intolerance" are common. Milk and other dairy products are a valuable source of calcium, which is needed especially for the construction of bones. Hence the use of milk should not be discouraged among children and adolescents without sound reasons (Seppo et al 2006). A quick and easily accessible genotype test is therefore valuable in clinical practice.

Furthermore, it is not known whether lactose consumption is harmful for people with genetic hypolactasia. Indirect evidence shows that there might be certain areas of increased risk. Studies concentrating on the possible risk of cancer have shown no definite increased risk of ovarian (Kuokkanen et al 2005), prostate (Torniainen et al 2007) or colon cancer (Rasinperä et al 2005) in the lactase non-persistent subjects. However, colon cancer needs further studies since in the Finnish study population there was statistically a significantly increased risk of colon cancer, yet in the Spanish or British study populations this was not the case (Rasinperä et al 2005).

2.1.2 Diagnosing adult type hypolactasia

The number of individuals suspected of having lactose intolerance is higher than the true prevalence of adult type hypolactasia (Jussila et al 1969; Johnson 1993; Vesa et al 1996; Caroccio et al 1998; Saltzman et al 1999; de Vrese et al 2001). Symptoms vary greatly in severity and depend on the amount of lactose ingested and on individual sensitivity, and may overlap with those of other gastrointestinal diseases such as IBS or diseases presenting with secondary lactose malabsorption i.e. coeliac disease or infection of the small intestine (Tamm 1994). It has been shown that lactose-restricted diets improve symptoms markedly, for example in irritable bowel syndrome (IBS) patients with primary lactose malabsorption, and reduce the number of visits to outpatient clinics (Bohmer 2001). Accurate diagnosis of adult-type hypolactasia, which is easily treatable by diet modification, is therefore cost effective and time-saving.

Diagnosis of adult-type hypolactasia has been based on indirect methods, the lactose tolerance test (LTT) or breath hydrogen test (BHT). These methods are time-consuming for the patients and need substantial assistance by medical personnel. Their sensitivity and specificity are not sufficient due to multiple factors affecting the results.

LTT is based on the measurement of the increase in blood glucose level after a 50 g oral lactose load. Blood samples are then taken at intervals of 20, 40 and 60 minutes. The time spent on collecting the blood samples is sometimes extended up to 120 min (Newcomer et al 1975). A blood glucose rise above 1.1 mmol/l is considered normal. Delayed gastric emptying can cause false positive results (Kern et al 1966; Newcomer et al 1966). LTT is also not reliable in diabetics (Lerch et al 1991). Furthermore, a variety of hormonal influences may have an effect on the result (Solomons 1981). Specificity of the LTT ranges from 77% to 96%, and sensitivity from 76% to 94% (Arola 1994).

BHT is based on the determination of hydrogen from expired air after an oral lactose dose of 50 g. Samples are taken at zero, and then at intervals of 15 to 60 min for 2 to 6 hours (Solomons 1981). Colonic flora has a crucial role since hydrogen production takes place in the colon and 14-21 % of the hydrogen produced is exhaled through the lungs (Levitt 1969). False negative breath hydrogen determinations can be detected in a fall of colonic pH and in active diarrhoea (Bond et al 1972; Perman et al 1981). Specificity of BHT varies from 89% to 100% and sensitivity from 69% to 100% (Arola 1994).

The gold standard of diagnosing adult-type hypolactasia, the invasive disaccharidase determination method requiring a jejunal biopsy, is not suitable for everyday clinical practice (Dahlqvist 1984).

Adult-type hypolactasia was shown in 1973 to be inherited as an autosomal recessive trait (Sahi et al 1973), and in 2002 a single nucleotide polymorphism C to T change 13910 base pairs from the 5'end of lactase gene trait in chromosome 2 was identified to associate with the lactase persistence/nonpersistence trait (Enattah et al 2002). Analyses of several hundred intestinal biopsies have demonstrated that the C/C_{-13910} genotype is associated with low lactase activity (<10 U/g/protein) and the C/T_{-13910}- and T/T_{-13910} genotypes with high activity (Enattah et al 2002; Kuokkanen et al 2003; Rasinperä et al 2004; Enattah et al 2008). In 2002 a commercial kit using this gene technology was applied in clinical practice (HUS-lab) and became available in primary care units. Since the test is based on molecular genetics, the result is valid lifelong and does not need to be repeated. However, only approximately 70% of the subjects having adult-type hypolactasia experience symptoms from lactose and 30% of subjects do not experience any symptoms from ingested lactose. (Carroccio et al 1998) Furthermore, in Finnish children the activity of lactase-phlorizin hydrolase does not on average decrease before school age; hence the test should be used with precaution in young children; but in the working-age population it gives valid information on the C/T_{-13910} genotype as a possible reason for lactose induced GI-symptoms (Rasinperä et al 2004).

2.2 Coeliac disease

2.2.1 History and clinical manifestations

Coeliac disease is a life-long autoimmune based entheropathy. Its history has been described since the second century B.C. (Aretaios). In the late nineteenth century the disease was described as a chronic indigestion resulting in loose stools, cachexia and distended abdomen. In 1952 a Dutch paediatrician, Dicke, discovered the harmful effects of barley, rye and wheat in coeliac disease, and as a result van de Kamer with his collegues conceived a strict gluten-free diet for the treatment of the condition. Paulley revealed in 1954 that small-bowel mucosal damage indicated coeliac disease, and a peroral apparatus for obtaining an intestinal biopsy for correctly diagnosing the disease was invented by Shiner in 1957. New, easier diagnostic methods have been applied for the screening of coeliac disease, yet the actual diagnosis is still based on intestinal biopsy.

In 1969 Van de Meer and Reunala with his colleagues revealed that coeliac disease is not only an intestinal disease but also has other extra-intestinal manifestations i.e. dermatitis herpetiformis (Van de Meer 1969; Reunala et al 1977). Later it was discovered that the disorder can also be asymptomatic or present only with mild forms of abdominal complaints or anaemia, tiredness and weight loss (Pare et al 1988; Corazza et al 1994; Mustalahti et al 1999; Tursi et al 2001; Green 2005; Haapalahti et al 2005). Isolated, subclinical malabsorption of iron, folic acid, calcium, vitamin D or B12 can also be present (Bode et al 1996; Kemppainen et al 1998; Dahele et al 2001; Kupper 2005). Neurological symptoms, reproductive disorders and an increased risk of malignancies, especially non-Hodgkin's lymphoma, have been described in connection with coeliac disease (Green et al 2003; Viljamaa et al 2006)

In clinically manifesting coeliac disease there is usually a secondary lactose malabsorption due to damage of the mucous villous system of the small intestinal membrane.

2.2.2 Diagnosing coeliac disease

Coeliac disease results from the interaction between genetic, environmental and immune factors. Gluten, found in barley, wheat and rye is necessary for igniting the disease. Genetic factors play an important role as the prevalence of coeliac disease is 11,5% among relatives of coeliac disease patients.

The correct diagnosis of coeliac disease in the early course of a symptomatic disease is important since the subtotal villous atrophy may cause nutritional deficiencies besides the gastrointestinal symptoms, with a reduction in the quality of life (Kemppainen et al 1998; Fabiani et al 2000; Howard et al 2002). In recent years the prevalence of undiagnosed coeliac disease is thought to be as high as 1:80 (Johnston et al 1997; Kolho et al 1998; Cook et al 2000; Mäki et al 2003)

Used in clinical practice for over fifty years, the intestinal biopsy is still the gold standard for diagnosing coeliac disease. A subtotal villous atrophy and crypt hyperplasia seen in the specimen are mandatory in settling the diagnosis (Interlaken criteria). Furthermore, T cells infiltration of the lamina propria, along with an increase in the number of intra-epithelial lymphocytes is a typical histological finding in coeliac disease (Silano et al 2009). An intestinal biopsy proceeded with a gastroscope is inconvenient for the patient and needs a specialist as well as assistant personnel to perform; hence the costs are also high. The non-invasive coeliac antibody tests have proved to be valuable in screening for coeliac disease and evaluating the need for villous biopsy. Additionally, the antibody test can be used in the guiding of dietary compliance (Sulkanen et al 1998; Collin et al 2002; West et al 2003). However, a jejunal biopsy should always be used for diagnosis when the symptoms are severe, even if the antibody tests are negative. The differential diagnosis to other mild forms of villous destructing conditions eg. infections of the jejunum or a specific food allergy is not always easy, and the specific serum antibodies for coeliac disease are useful in confirming the diagnosis.

The coeliac antibodies are produced in untreated disease in the intestinal mucosa (Marzari et al 2001). The production of antibodies additionally results in coeliac disease-specific antibodies in the circulation. The antibodies are mainly of IgA-class. Of these, the autoantibodies i.e. reticulin antibody (ARA), endomysium antibody (EMAb) and tissue transglutaminase antibody (tTG-ab) are formed against the patient's own tissue structures, endogenous antigens. Endomysium, literally meaning within the muscle, is a layer of connective tissue that ensheaths a muscle fibre and is mostly composed of reticular fibres and has specific endomysium antigens. tTG is a calcium-dependent enzyme and has specific antigens. It is stored mainly intracellularly and released to extracellular space upon mechanical and inflammatory stress (Bergamini et al 1999). EMAb and tTG-ab are presently used in clinical practice. These antibodies should always be measured together with serum IgA, since false-negative results can be obtained in patients with selective IgA deficiency (Collin et al 1992; Cataldo et al 1998). The EMAb test is based on immunofluorecence giving a qualitative result (Ladinser et al 1994), where as tTG-ab is based on a quantitative result with ELISA (Dietrich et al 1997).

In clinical laboratory practice, human umbilical cord is currently used as an antigen. The sensitivity of IgA-class EmAb test varies from 75% to 100%, but in most studies is above 92%. Specificity varies accordingly, but is in most studies close to 98%. The sensitivity of IgA-class tTG-ab is above 90 % in the majority of studies published and specificity 95-100% (Salmi T 2006). In patients with selective IgA deficiency the IgG-class coeliac antibodies (S-EMAbG and S-tTGAbG) are used in clinical practice (www.yhtyneetlaboratoriot.fi). Their sensitivity and specificity are almost similar to IgA-class antibodies (Collin et al 1992; Cataldo et al 1998). The sensitivity and specificity of these antibody tests varies according to the transglutaminase protein, which is used as an antigen. It is most important to have a profound knowledge of the commercial antigen product used since the test result can vary accordingly. A new rapid test for coeliac disease, based on Finnish-Hungarian innovation, can even be used at home. The test kit is easy to use and the clinical accuracy is similar to the conventional antibody-based tests (Korponay-Szabo et al 2007; Raivio 2008).

Coeliac patients must have an HLADQ2 or DQ8; however, these two genotypes are necessary but not sufficient for coeliac disease since 40 % of healthy Caucasians have these same heterodimers (Mäki et al 2003). Thus, the HLA genotyping is not used in normal clinical practice when diagnosing coeliac disease.

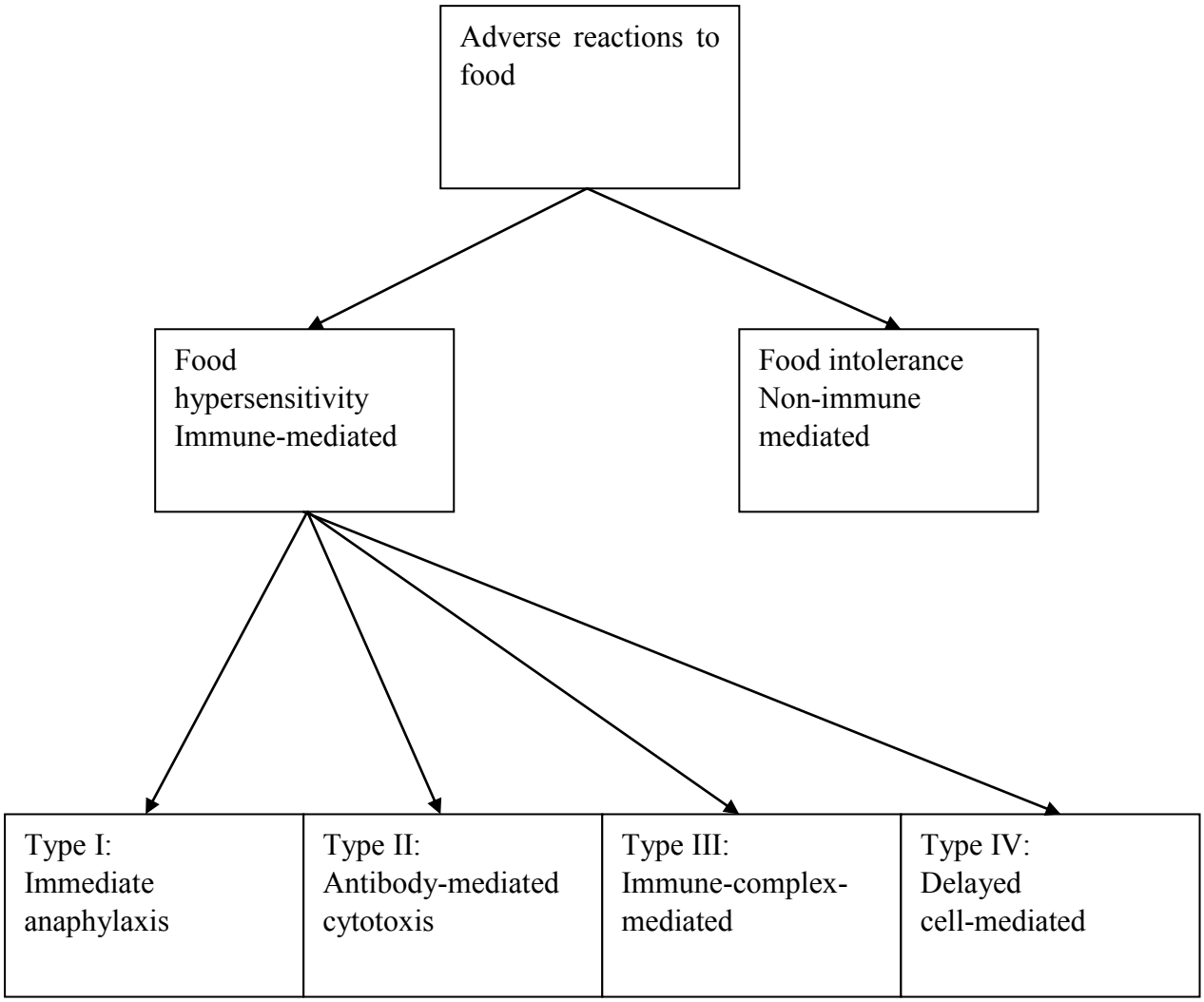
ARA or gliadin antibodies (AGA) are no longer used in clinical practice due to their low sensitivity and specificity. However, the new deamidated gliadin peptide (DGP) antibodies might in future prove to give reliable results (Kaukinen et al 2007; Rashtak et al 2008)

2.3 Hypersensitivity to milk protein

2.3.1 History and clinical manifestations

Adverse reactions to food are classified as non-immune mediated food intolerance or immune mediated food hypersensitivity (Bruijnzeel-Koomen et al 1995). Food hypersensitivity or food allergy is triggered by an aberrant immune response caused by ingestion of dietary antigens. Allergic reactions are traditionally classified under four types of hypersensitivity reactions (Britton et al 2002; Male et al 2002). More than one mechanism may be involved in allergic reactions, but the most plausible mechanisms are IgE mediated (Type I) immediate anaphylactic reactions. Antibody-dependent cytotoxic (Type II) hypersensitivity is an important part of the body's normal humoral immune response (Male 2002). In this type of hypersensitivity, IgG and IgM antibodies identify cell-surface antigens and activate the complement system and damage the cell. Immune-complex-mediated (Type III) hypersensitvity is an effector-cell and complement mediated tissue damage (Hay et al 2002). Delayed cell-mediated (Type IV) hypersensitivity reaction takes more than 12 hours to develop (Britton 2002). In a Type IV reaction, T cells identify antigens after which they produce cytokines and other soluble factors and hence mediate the hypersensitivity reaction. It is not known, whether all four types of reactions are involved in the pathogenesis of food allergy.

Figure 1 *Different types of food hypersensitivity*



The symptoms of food allergy may appear in the skin, in subcutaneous tissue, in the GI-system or in the respiratory tract. Typical cutaneous symptoms of food hypersensitivity are rash, urticaria, atopic dermatitis and, more seldom, angio-oedematic reaction. Typical gastrointestinal symptoms are diarrhoea, pain and vomiting. Asthmatic, eye and nasal symptoms also do exist (Nermes et al 2009).

Cow's milk allergy (CMA) is IgE mediated in 57-64% of allergic infants under the age of one year (Vanto et al 1999; Saarinen et al 2000). The majority of paediatric patients have symptoms from more than one organ system: 50-60% have cutaneous, 50-60% gastrointestinal and 20-30% respiratory symptoms (Host 2002). However, the prognosis of CMA in infancy is good with a remission rate of 85-90% by the age of three (Host et al

1990; Saarinen et al 2005). In infancy, the non-IgE mediated delayed type reactions to cow's milk recover the quickest (Vanto et al 2004).

CMA in school-aged children and adults is not IgE mediated but more like a new form of delayed-type gastrointestinal cow's milk hypersensitivity, also known as cow's milk entheropathy (Bengtsson et al 1997; Ulanova et al 2000; Kokkonen et al 2001). The mucosal changes in cow's milk enteropathy are clearly different of those in coeliac disease (Kokkonen et al 2001; Turunen et al 2004) or small children with food allergy.

The study among young Finnish adults showed a high serum reactivity to milk protein in subjects clinically nontolerant to milk but with normal lactose tolerance (Pelto et al 1998), and this finding was confirmed in a larger study (Pelto et al 1999) implying that serum reactivity, i.e. capacity of antigen-antibody complexes to activate phagocytes, may have a role in adult milk hypersensitivity. Only occasional case reports of IgE-mediated cow's milk allergy in adulthood exist.

2.3.2 Diagnosing cow's milk allergy

No single laboratory test is diagnostic for either delayed- or immediate- type of cow's milk allergy. Hence, the diagnosis is still based on strict elimination and challenge procedures. In patients with delayed reactions, a placebo-controlled food challenge would be the most reliable method, but it is clinically not practical (Bindslev-Jensen et al 2004). Especially in adults, the main gastrointestinal symptoms of cow's milk hypersensitivity are difficult to distinguish from the ones caused by primary or secondary hypolactasia.

Patients suffering from IBS often relate their GI-symptoms to milk (Hillilä et al 2007). Dyspepsias can also be related to milk (Pajala et al 2005), either to hypolactasia or hypersensitivity to milk. Zuo et al 2007 in China studied serum food antigen-specific IgG and IgE antibodies in patients suffering either from IBS or functional dyspepsia (FD), comparing them to the group of healthy controls. They found no difference in the titres of food specific IgE antibodies between the groups. The serum food specific IgG antibody titres were higher among the subjects belonging either to the FD- or IBS-group compared to the healthy controls, but there was no correlation between the titre level of the antibody and the symptom severity (Zuo et al 2007).

In early infancy the presence of a specific elevation of IgE antibodies to cow's milk and either a skin prick or patch test for cow's milk may be diagnostically valuable. Skin prick or patch tests and milk protein IgE are rarely useful in adults (Norgaard et al 1992). The roles of food specific and especially milk specific IgG and IgA are currently being studied, but so far there is no consensus on their usefulness in clinical practice.

3 Aims of the study

The aims of this study were:

To study the prevalence of milk-related symptoms among patients with C/T_{-13910} genotypes and the usefulness of the genetic test of adult-type hypolactasia in primary health care

To evaluate undiagnosed coeliac disease, the most common cause of secondary hypolactasia; its prevalence and correlation to clinical symptoms.

To clarify hypersensitivity to milk protein; the role of cow's milk protein IgE in adults.

To assess the role of cow's milk protein IgG and IgA in milk hypersensitivity in adults.

4 Subjects and methods

4.1 Study subjects

4.1.1 Study No. I: C/T$_{-13910}$ genotype-testing

This study included 1900 working age adults (18-64 years) from the capital area of Finland, who visited a health care centre laboratory for different reasons in spring 2004. Four different health care centres in the city of Espoo and the outpatient clinic of the Military Hospital Tilkka took part in the study. The targeted size for the study was 2000 subjects, but due to understaffed laboratory personnel the study was ended prematurely with only 1900 subjects. The decision was made not to continue the study later (after the summer holidays) in order to minimize the seasonal variation on the study results. All the samples and questionnaires were collected in a three-month period from February to May 2004.

An information brochure about the study was put up on the walls of the laboratory waiting room. The questionnaires and the written consent forms were available in the waiting room, and the patients were advised to fill in the questionnaires while waiting for a laboratory test. The volunteers completed a questionnaire on gastrointestinal symptoms and consumption of dairy products. The laboratory personnel provided verbal information about the study, took the blood samples and collected the written consent forms together with the questionnaires. The blood samples were sent for analysis to the study laboratory of the University Hospital in Helsinki. The questionnaires were collected weekly by the dissertee. The answers to the questionnaires were put into Excel format, after which they were statistically analysed.

Of the total study group, 21% of the subjects belonged to the youngest age group of 18-35 years, 34 % were aged 36-51 years and 45 % 52-64 years. 73% of the participants were women (Table 2).

Table 2 *Demographic parameters of the study group*

	Men	Women	Total
n (%)	509 (27)	1391 (73)	1900 (100)
Age distribution • 18-35 years • 36-51 years • 52-64 years	 148 (29) 166 (33) 195 (38)	 244 (18) 489 (35) 658 (47)	 392 (21) 655 (34) 853 (45)
Questionnaires completed	505 (99)	1380 (99)	1885 (99)
Gastric symptoms previous 3 months	334 (66)	1147 (83)	1481 (79)
Milk-related GI-symptoms	133 (26)	623 (45)	756 (40)
Food-related GI-symptoms	254 (50)	926 (67)	1180 (62)
Laboratory visit • Health check-up (or other reason) • Abdominal complaints • Nongastro-intestinal disease • No answer	 292 (58) 76 (15) 116 (23) 25 (5)	 703 (51) 272 (20) 329 (24) 87 (6)	 995 (53) 348 (18) 445 (24) 112 (6)
Drinking milk n (%) • Yes • No • No answer	 237 (47) 264 (52) 8 (2)	 392 (28) 975 (70) 24 (2)	 629 (33) 1239 (65) 32 (2)

4.1.2 Study No. II: undiagnosed coeliac disease and its clinical symptoms

For this study, we included the serum samples of the total cohort of 1900 subjects. The samples were screened for coeliac disease and the answers to the questionnaires evaluated accordingly. The subjects with an elevated serum transglutaminase and endomysium antibody titres, and hence considered as possibly having undiagnosed coeliac disease, were contacted by phone and requested to have a gastroscopy done if possible. The telephone interview also included a questionnaire of possible gastrointestinal symptoms related to cereal, the presence of coeliac disease in close relatives and other coexistent diseases of the study subjects. The results of the gastroscopies and biopsy samples were also accordingly requested by phone.

4.1.3 Study No. III: the role of cow`s milk protein IgE in adults

This study included all 756 participants of the original study group of 1900 patients attending the laboratory who reported suffering from milk-related gastrointestinal problems. A control group of 101 subjects was randomly selected from those reporting no milk-related symptoms (n=638). Of the 1885 participants returning the questionnaire, 491 did not answer the question on milk-related gastrointestinal symptoms and were excluded from the selection.

4.1.4 Study No. IV: the role of cow's milk proteins IgG and IgA in milk hypersensitivity in adults

For this study, 400 subjects (198 women and 202 men) were randomly selected from a total of 1900 adults attending laboratory investigations in primary care. All participants had completed a questionnaire on abdominal symptoms and dairy consumption. Twelve samples were excluded due to insufficient amount of sera. Thus, the study group comprised 388 adults (aged 18-64 years, mean age 40 years) of whom 119 reported experiencing gastrointestinal symptoms from milk, and 198 reported having no milk-related symptoms.

4.2 Methods

4.2.1 Questionnaires

All 1900 participants were given a questionnaire to complete while waiting for the laboratory, or immediately after the blood sample had been taken. 1885 questionnaires were returned (99%). Each participant provided their name, address, social security number, reason for attending the laboratory (four different categories: gastrointestinal

symptoms; a check-up due to a previously diagnosed disease; health check-up; other reason). They were asked whether they had had gastrointestinal symptoms in the previous three months and during the preceding week, and if they suspected the symptoms to be related to food and especially to milk ingestion. We also enquired their consumption of dairy products and the different types of dairy products, and the relation of dairy consumption to GI-symptoms. The previous history of atopy and hypersensitivity to common inhalant and food allergens as well as history of diagnosed gastrointestinal diseases (coeliac disease, inflammatory bowel disease, gastric ulcer, *Helicobacter pylori* positivity, cancer of gastrointestinal tract and cholecystectomy); the family history of gastrointestinal diseases was also requested. Additionally, we recorded the current weight and height of the subjects. The total number of items asked was 24, and additionally it was possible to give unstructured information. In order to test the validity and accuracy of the questions, the questionnaire was pre-tested among a small number of working-age healthy adults.

4.2.2 Laboratory and functional analyses

4.2.2.1 Genotyping for C/T$_{-13910}$

All 1900 blood samples were sent to the Helsinki University, Department of Clinical Genetics for analysis of the C/T$_{-13910}$ genotype of the subjects. DNA of the study subjects (n=1900) was isolated from peripheral blood samples.

The C/T$_{-13910}$ single nucleotide polymorphism was analysed using the solid-phase minisequencing method (Syvänen et al 1990) which is based on the detection of tritium-labelled T-13910 and C-13910 alleles in the PCR reaction and measurement of their ratio using scintillation counter that directly reflects the ratio between the two sequences in the original sample.

4.2.2.2 Analyses for undiagnosed coeliac disease

All 1900 blood samples were analysed for coeliac disease. Tissue transglutaminase antibody of IgA class, TG2A was determined for all study subjects. The HLADQ2 and DQ8 genotypes, which are the major HLA genotypes associating with CD, (Mäki et al 2003) were determined in each patient with a TG2A level above the cut-off limit (8 %), and in those who reported that they were CD patients. Antiendomysial antibodies of IgA class, EmA (Kolho et al 1998) were determined in each TG2A positive case. Total serum IgA was determined nephelometrically (Kolho et al 1997; Sulkanen et al 1998). Nutritional status was determined by the subjects' body mass indexes (BMI) and measuring iron (S-Fe), transferring receptor (S-TfR), and folate in their serum. The established reference ranges were 9-34 umol/l for S-Fe, 0.9-2.3 mg/l for S-TfR and 4.5-34 nmol/l for folate. The cut-off value for underweight individuals was BMI <18.5 kg/m^2 and for overweight individuals BMI's of 24 kg/m^2 for men, and 25 kg/m^2 for women (Cole et

al 2000). Fourteen subjects who were positive for TG2A and EmA were contacted personally to re-check their patient history (see above) and to give them advice about a gastrointestinal biopsy.

4.2.2.3 Analyses for milk protein IgE

The Pharmacia CAP System was used for screening of specific IgE against major food allergens (wheat, codfish, peanut, egg (ovalbumin), soy-bean, cow's milk) in a total of 857 subjects. Values equal to or higher than 0.35 IU/l were considered positive. Those with a positive total screen were further screened for specific IgE to untreated skimmed milk (f2; milk-IgE) and boiled milk-IgE (f231; Pharmacia ImmunoCap System).

All thirteen individuals with a positive milk-IgE test were invited to an open milk challenge test, which was performed in the Skin and Allergy Hospital under strict hospital control and surroundings. A total amount of 570 ml of low-lactose and low-fat milk was given orally in a 65-minute period and the challengees were followed-up at hospital for two hours. The skin reactions and gastrointestinal symptoms were registered. The follow-up was continued for a further 24 hours by the subjects.

4.2.2.4 Analyses for milk protein IgA and IgG

The milk protein IgG and IgA were measured as described from 400 randomly picked samples by ELISA using an adapted infant formula to coat the microtitre plates, and values were expressed as % of the standard with a very high titre of cow's milk antibodies (Savilahti et al 1993). The major antigen in the formula was casein.

IgG antibodies to *Helicobacter pylori* were measured from all 1900 participants with an in-house enzyme immunoassay as previously described (Oksanen et al 1998). The lower limit for raised titres was 700 with a sensitivity of 99% and specificity of 93% as compared to histology (Oksanen et al 1998).

4.2.3 Statistical analyses

Statistical analyses were conducted using Tixel (version 8.1), which is a VBA-programme for Excel. Descriptive analyses were conducted with simple logistic regression. Proportions were compared by using Chi-squared tests with continuity correction. Also Kruskall-Wallis test, Spearman Rank Correlation, Mann-Whitney, Fisher's exact test, and ANOVA were used for analysing the results when appropriate. Significance was set at $p<0.05$.

5 Results and discussion

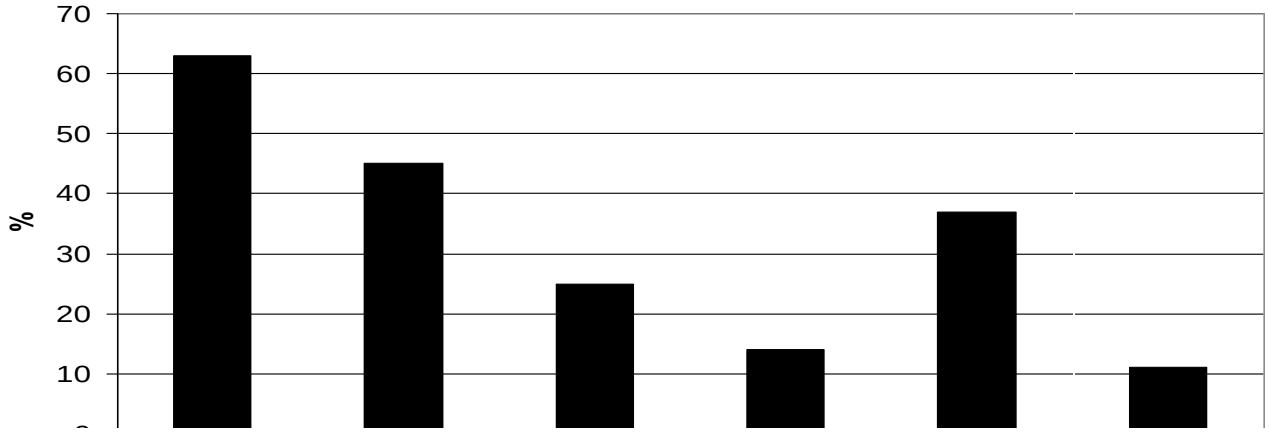
5.1 Adult-type hypolactasia

In the first study, we evaluated the correlation of the C/T$_{-13910}$ genotypes with reported milk induced GI-symptoms and the clinical usefulness of the human genotype based C/T$_{-13910}$ test in diagnosing adult-type hypolactasia. The recessively inherited C/C$_{-13910}$ genotype has been shown to correlate with low lactase activity (<10 Ug/l), whereas the C/T$_{-13910}$ and T /T$_{-13910}$ genotypes have been shown to possess a higher lactase activity: C/T$_{-13910}$ genotype > 30 Ug/l and T /T$_{-13910}$ genotype >50 Ug/l (Järvelä et al 2005). Adult-type hypolactasia is the most common enzyme deficiency affecting 17% of the Finnish population (Sahi 1994). In this study the prevalence of lactase non-persistence was 18%, reconfirming the previous figures of lactase non-persistence in the Finnish population. There was no difference in genotype distribution among males and females, or different age groups.

The study participants

Of the subjects giving a blood sample for the study, the response rate to the questionnaires was extremely high, 99% (1885/1900). The majority of the participants were women (73%), most likely confirming the fact that women are keener to volunteer for different types of studies (West et al 2003). It might also imply that women visit health care centre laboratories more often than men. However, this female predisposition may somewhat bias the results. The total number of laboratory visitors that took part in the study as well as the number of subjects who refused to participate was not evaluated; nor the number of male or female visitors to the laboratory in the study period. 80% of the participants reported having had GI-symptoms in the previous three months, which accords with the study of Drossman et al in the American population (Drossman et al 1993). The result shows that persons without at least occasional GI-symptoms are a rarity. The implication is also that person having GI-symptoms would be more likely to take part in the study, which could have a biasing effect on the results. Over 60% of the participants related their GI-symptoms to food, and especially to milk *(Figure 2*).

Figure 2 *The self-reported correlations of GI-symptoms to various food substances (modified from study No. I)*



The reason for a laboratory visit was associated with GI-symptoms in only 19% of the subjects, and of them 24 % had the C/C$_{-13910}$ genotype, a higher frequency of hypolactasia than in an unselected Finnish population. The connection of GI-symptoms as a reason for a laboratory visit and the C/C$_{-13910}$ genotype was statistically significant compared to the persistent genotypes C/T$_{-13910}$ and T/T$_{-13910.}$ Also the GI-symptoms in the previous three months were reported significantly more often in the C/C$_{-13910}$ genotype group; the frequency of GI-symptoms was also higher than in the persistent genotype groups, but this difference was not statistically significant.

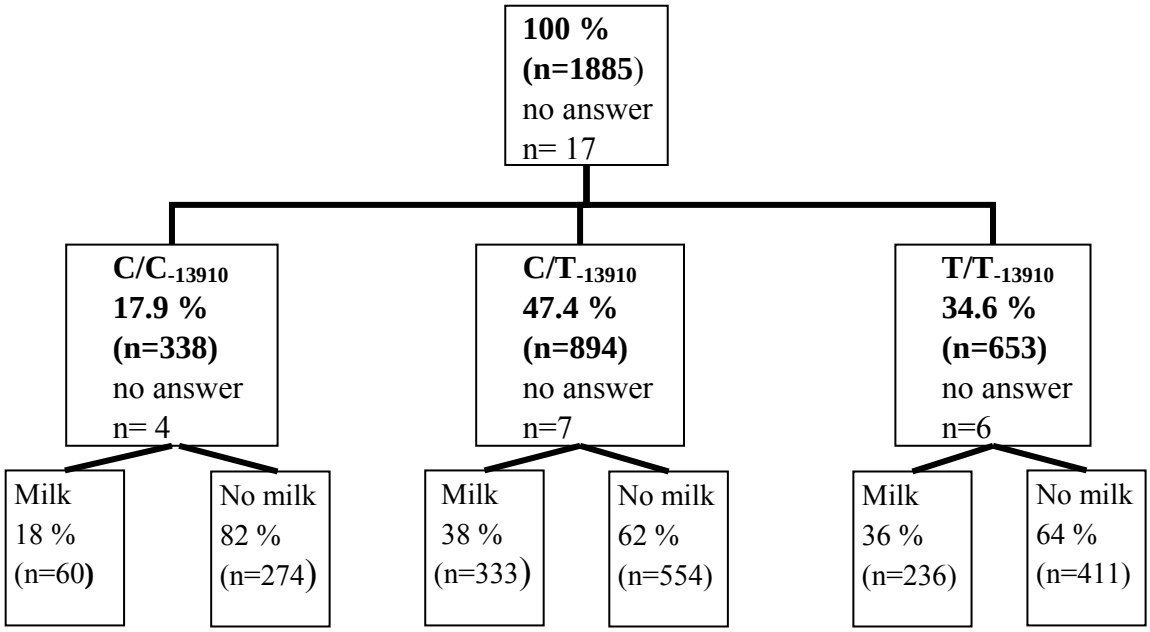
Of the GI-symptoms, only flatulence was significantly more frequent in the C/C$_{-13910}$ genotype group compared to the lactase persistent groups. This result differs from that of Jussila et al 1969, since according to them all the three above-mentioned symptoms are more frequent in subjects with hypolactasia. All the three above-mentioned symptoms of flatulence, diarrhoea, and bloating were common in the study group, since 40-60% of the subjects reported having experienced them in the previous three months. This supports the results of Böhmer et al 1996 and 2001, and Hillilä et al 2004 showing the symptoms of lactose intolerance as not being different from the ones of functional GI-symptoms.

Cow's milk consumption as a drink

Almost half of the study population reported experiencing gastrointestinal symptoms after drinking milk (*Figure 2*), and one fourth of the participants reported symptoms from food containing milk. Only 18% (60/338; $P<0.01$)) of the subjects with the C/C$_{-13910}$ genotype of adult-type hypolactasia reported drinking milk with meals, which is significantly less ($P<0.01$) than those with the lactase persistent genotypes C/T$_{-13910}$ (38%; 333/894) and

T/T$_{-13910}$ (36%; 236/653; Figure 3). The fat content of the milk was not investigated, but in Finland low-fat or fat-free milk are typically consumed among milk-drinkers (www.maitojaterveys.fi). Only occasionally is full cream milk consumed as a drink. According to the questionnaire, very few people used low lactose content milk as a drink (89/1779).

Figure 3 *Flow-chart of cow's milk consumption as a drink in different genotypes (modified from study No. I)*



Only 9% (29/338) with the C/C$_{-13910}$ genotype of adult-type hypolactasia consumed milk daily and reported no symptoms from milk. However, one third (18/60) of the milk drinkers with the C/C$_{-13910}$ genotype did not answer the question about milk-related symptoms. Most participants with the C/C$_{-13910}$ genotype reported GI-problems from milk (69%; 190/274) and did not drink milk. The respective number of people with lactase persistence genotypes who reported milk-related problems and did not drink milk was 54% (299/554) for the C/T$_{-13910}$ genotype and 50% (207/411) for the T/T$_{-13910}$ genotype. The result was statistically not significant, yet implies that people with the C/C$_{-13910}$ genotype tend to consume less milk than the two lactase persistent genotypes, which was seen among children in the studies of Rasinperä et al 2006. In a recent study among Finnish children from childhood to young adulthood, the subjects with the C/C$_{-13910}$ genotype had consumed less milk since childhood. However, the consumption of other

milk products did not differ between the persistent and non-persistent genotypes (Laaksonen et al 2009).

Variation in tendency of experiencing GI-symptoms

The tendency to experience GI-symptoms from milk varies due to several aspects. The colonic microbiota is variable (Egert et al 2006) and the individual sensitivity to feel distension of the colon and to sense discomfort varies (Vesa et al 1998). The meal content also has an important effect on emptying of the stomach, and thereby affects the lactose load in the intestine (Martini et al 1988; Vesa et al 1997). Accordingly, the recognition of the possible link between milk consumption and abdominal symptoms is not always easy (Suarez et al 1995). According to previous studies, subjects having C/T$_{-13910}$ genotype have a lower lactase level than subjects with T/T$_{-13910}$ genotype (Enattah et al 2002; Kuokkanen et al 2003; Rasinperä et al 2004; Enattah et al 2008). In this study the consumption of milk, or the subjective milk-related symptoms, did not differ in these two persistent genotype groups.

Most lactose malabsorbers seem to tolerate small amounts of milk, especially during meals (Vesa et al 1996 and 2000). The amount tolerated has in most cases been shown to be 20 g of lactose (Vesa et al 2000). In this study we did not ask the amount of milk the individuals are able to consume without having GI-symptoms, but requested what kind of dairy products the study subjects consumed, and the possible effects of the products on their GI-symptoms. It was notable that even though almost half of the study population suspected milk related GI-problems, the consumption of low lactose content milk was minimal.

Cheese caused gastrointestinal symptoms in 11% of the participants according to their own judgement: for 17% of those with the C/C$_{-13910}$ genotype, 10% of those with the C/T$_{-13910}$ genotype and 9% of the ones with the T/T$_{-13910}$ genotype ($P < 0.05$). This was somewhat surprising since cheese loses most of its lactose concentration by ripening (Fox et al 1990). Among all participants, 14% experienced symptoms from cereal or bread, and 37% from ingested fat (*Figure 2*), and these symptoms were not related to the genotype of adult-type hypolactasia.

Comparison of the C/T$_{-13910}$ gene test to LTT

Among the study population, 15% (245/1649) reported having had a positive lactose tolerance test (LTT) earlier. A previous, pathological LTT was reported by 19% (64/341) of participants with the C/C$_{-13910}$ genotype, by 10% (89/901) with the C/T$_{-13910,}$ and by 14% (91/658) with the T/T$_{-13910}$ genotype. Five out of these 180 subjects with the C/T$_{-13910}$ or T/T$_{-13910}$ genotype with a pathological LTT reported a previously diagnosed possible secondary cause for hypolactasia i.e. coeliac disease. An undiagnosed coeliac disease in two other participants of these 180 subjects was suggested due to an elevated level of transglutaminase antibodies in their sera. The relatively high percentage of subjects with either C/T$_{-13910}$ or T/T$_{-13910}$ genotype with no secondary cause for hypolactasia having a positive LTT imply that LTT, a commonly used method for diagnosis of lactose intolerance, produces high numbers of false positive test results, as has been previously observed (Arola 1994). Since the study subjects were only asked about any earlier positive

diagnosis of lactose intolerance, the extent of subjects with the C/C$_{-13910}$ genotype who might have had a negative result for their LTT is not known.

The cost benefit of the DNA-based gene test to the LTT was evaluated by Piirainen et al in 2007. They concluded that compared to the gene test the LTT was ten Euros more expensive per patient. Hence not only the specificity, but also the cost benefit is better for the C/T$_{-13910}$ gene test.

Main results

To conclude the results and discussion of adult-type hypolactasia, the following are the main findings that emerged from our study: gastrointestinal symptoms are more common among adults with the C/C$_{-13910}$ genotype of adult-type hypolactasia than among those with genotypes of lactase persistence. This was seen even though individuals with the C/C$_{-13910}$ genotype had restricted their milk consumption. Genotyping for C/T$_{-13910}$ polymorphism is a practical means for defining adult-type hypolactasia.

5.2 Coeliac disease

Prevalence of coeliac disease

Among the study group of 1900 subjects, 33 subjects had an elevated TG2A (> 8 units) level. One of these subjects had a recent diagnosis of CD, but all the others with an earlier diagnosis of coeliac disease were negative for TG2A. We thus detected 32 suspected cases of coeliac disease with screening of TG2A. To confirm our suspicion we measured EmA titres of these 32 subjects and found 14/32 increased (>1:50) titres. 18/32 subjects with a normal EmA titre were excluded from the group of screen-detected coeliacs. All subjects in the series had normal IgA values. All 14 screen-detected coeliacs were positive for HLADQ2 or HLADQ8 that associate with coeliac disease.

22/1885 subjects (1%) reported having had a coeliac disease diagnosed earlier. Of these, 16 had undergone gastrointestinal biopsy and in 6 cases the information was not given. 18/20 (two blood samples were not available) of these cases had either HLADQ2 or HLADQ8 genotype. Hence the total prevalence for coeliac disease was 1:53 when the screen-detected and all the diagnosed coeliacs were included. This is higher than in any earlier published studies dealing with unselected populations (1:133 in Fasano et al 2003; 1:83 in West et al 2003; 1:67 in Mäki et al 2003 except for Lohi et al 2008, who found the prevalence of 2 %). The criteria for a positive diagnosis of coeliac disease were strict since elevated titre of both TG2A and EmA was required. This implies that the true prevalence of coeliac disease may be even higher. The cohort screened for CD included a 73% majority of women probably due to the fact that women are in the majority among voluntary screening participants (West et al 2003). Gender-specifically undiagnosed CD was detected in 1:116 (12/1391) of the women and 1:255 (2/509) of the men. Previous diagnosis of CD was reported among 1:77 (18/1391) of the women and 1:127 (4/509) of the men, ending up in a total prevalence of 1:46 for CD in women and 1:85 for men. Coeliac disease is estimated to be more common among women than among men (Green

2005), which was also the case in our study. However, there are several reports showing no sex difference in the prevalence of CD (Hin et al 1999; Cook et al 2000; Collin et al 2002).

The 14 screen-detected undiagnosed CD patients were personally contacted by the author. 6/14 reported having milk related GI-symptoms, and only one considered cereal as being causative of her GI-symptoms. Ten patients were willing to undergo gastrointestinal biopsy. The biopsy was positive in 8 subjects, the result was not available in two subjects, one person refused further investigations, and one person was unsure about undergoing gastroscopy. Two patients could not be reached. Thus the findings in biopsies were consistent with the positive serum screening, and confirmed the diagnosis of CD.

Comparison of screen detected and previously diagnosed coeliacs

The BMIs of the screen-detected coeliacs were compared to the ones with a previous diagnosis of coeliac disease on a gluten-free diet. The mean BMI of the screen-detected coeliacs was 27.1 (range 20.1-41.5), which did not differ from the ones with previously diagnosed CD (mean 24.2; range 17.9-32.0). One female with an established diagnosis of CD was found to be underweight. A total of 7/14 screen-detected patients were overweight (four of them were obese), and 9/21 with a known CD were overweight (two cases were obese). The difference in the number of overweight subjects between these two groups was not significant. However, one third of the screen-detected CD patients were also obese. There is little data on obesity in CD (Oso et al 2006). It is important to note that a significant number of screen-detected patients are of normal weight or even overweight, and not underweight (Cook et al 2000; West et al 2000; Haapalahti et al 2005).

The indication for blood sampling was a health check-up at primary care centres in the majority of patients with undiagnosed CD (9/14). In two cases the reason was abdominal complaints, the main symptoms being flatulence and diarrhoea. Three responders (3/14) reported abdominal symptoms on eating cereals. This might suggest that self-experienced symptoms are vague in the majority. Milk consumption did not differ between those with a previous diagnosis of CD (no milk 19/22) or undiagnosed CD (no milk 9/14) significantly showing the majority in both groups to be non-milk drinkers. Those with a previous diagnosis of CD reported more heartburn than those with an undiagnosed CD, but no further difference in the other gastrointestinal symptoms was discovered.

Adult type hypolactasia based on a lactose tolerance test had been diagnosed in one screen-detected subject. One patient reported a previous suspicion of CD, but there had been no definite diagnosis and no follow-up. Of the screen-detected CD patients, only 7 subjects reported a concomitant diagnosis of another chronic disease. Among relatives of the screen-detected CD patients, one person reported having a mother with CD.

Undiagnosed CD may be associated with a lack of nutrients

Nutritional deficiencies were observed in 50% of the screen-detected CD patients; especially the levels of iron and folate in serum were lower when compared to those with a previous diagnosis of CD and on a gluten-free diet (*Table 3*). A deficiency in iron was rare, contradictory to symptomatic adult patients with CD (Collin et al 2005). The earlier result that 16% of the screen-detected CD patients may present with anaemia (West et al

2003) is in line with the findings of this study. Serum folate was below the established reference range in half of the screen-detected CD patients, which might imply a subtotal villous atrophy (Kemppainen et al 1998). Blood haemoglobin or serum calcium was not analysed.

In screen-detected children with CD, nutritional impairments such as a decrease in serum folate level and indicative findings for iron deficiency, were present in one third of the study subjects (Haapalahti et al 2005). This is less frequent than present adult study shows. Undiagnosed CD may predispose to impaired bone density (Mustalahti et al 2001); it might also be associated with a lack of other nutrients such as zinc (Kemppainen et al 1998; Viljamaa et al 2005), and vitamin B12 (Dahele et al 2001). Likewise, undiagnosed CD may be associated with fatigue and other unspecific symptoms even in the absence of nutritional impairments (Hin et al 1999; Sanders et al 2003). But the presence of such unspecific symptoms was not requested.

Table 3 *Comparison of nutritional parameters of screen-detected and previously diagnosed coeliacs*

Diagnosis	Coeliac disease Gluten-free diet	Screen-detected coeliac disease
Gender (male/female)	4/18	2/12
Median age Years (range)	44 (23-64)	48 (21-60)
BMI : Median (range) BMI >30	24.6 (17.9-32) 1/1	25.7 (20.1-41.5) 0/4
S-Fe below the reference range < 9 umol/l	1/21	2/9
S-TfR above the reference range > 2.3 mg/l	0/21	1/9
S-Folate below the reference range < 4.5nmol/l	1/21	6/12

Main results

Undiagnosed coeliac disease was found to be even more common than earlier studies had shown, probably due to the over representation of females among the adult population undergoing screening. Nutritional deficiencies were present in half of the screen-detected patients even though the great majority considered themselves to be healthy. Nutritional impairments were mild, a decline in serum folate levels being the most common.

There was though, no clear correlation between the GI-symptoms or the BMI and undiagnosed coeliac disease. However, the result showing nutritional impairments in every other screen-detected CD patient suggests that screening for CD should be actively implemented for the working-age population. A further, more recent study from Finland has shown that a gluten-free diet should be initiated after a positive serological screening

for coeliac disease in order to prevent gradual mucous villous destruction (Kurppa et al 2008). Long-term dietary compliance and the quality of life is good among screen-detected CD adults (Viljamaa et al 2005), which also encourages screening for coeliac disease in risk groups.

5.3 Hypersensitivity to cow's milk

This section of the study focused on milk protein IgE, IgA and IgG mediated reactions to milk in an adult population. Due to the large number of study subjects (1900), two different sub-groups were formed from the original study group. Hence the results of the IgG/A and IgE groups must be evaluated separately.

5.3.1 Milk protein IgE

When analysing milk protein IgE, it was found that 1.5 % (13/857) of the previously described study group had an elevated milk protein IgE value (>0.35 IU/l). In women, the percentage of positive reactions to milk-IgE was 1.3% (9/673), and in men 2.2% (4/184). However, the difference was not statistically significant. All milk protein IgE positive subjects belonged to the age group of 35-49 years. Milk-IgE for boiled milk was positive in 3 subjects, one of them being negative for standard milk-IgE antibodies.

No correlation was found with milk drinking and IgE antibodies for milk. The prevalence of milk-IgE was not statistically different between those with milk-related symptoms and those with no such symptoms (1.6% / 1 %). Those reporting no milk-related problems used milk as a drink more often (58/101; $p<0.001$). Only one subject positive for cow's milk-IgE antibodies reported no milk-related symptoms.

An open food challenge

All 13 milk-IgE positive adults were contacted by phone, and all 9 who could be traced accepted the invitation to a cow's milk challenge test. The aim was to test the clinical relevance of milk-IgE positivity by performing a food challenge with milk. In addition, the subject negative for milk-IgE, but positive for boiled milk- IgE agreed to testing. Thus, ten subjects in all took part in the food challenge. These subjects had reported milk-related GI-symptoms except for one person. An open milk challenge was arranged at the Skin and Allergy Hospital of Helsinki University. All subjects experienced abdominal discomfort and bloating during the ingestion of 570 ml of milk. One subject reported diarrhoea immediately after the ingestion, but none of the subjects developed skin symptoms. The open protocol used in the milk challenge test may have had a psychological impact on the prevalence of abdominal symptoms. The decision to challenge only the subjects with an elevated concentration of milk-protein IgE, and not to include milk-protein IgE negative subjects, was based on the reports showing that immediate reactions are likely to occur

only in those with IgE antibodies to the challenged food allergen (Sampson 2001; Ewan et al 2005; Mansueto et al 2006). A control group with no IgE antibodies would not have changed the negative results of the challenge test.

The milk challenge was performed with low lactose milk to avoid symptoms related to lactose malabsorption. One subject with the genotype C/C_{-13910} associated with low lactase level developed diarrhoea immediately after the milk ingestion, and the reaction was thus considered hypolactic rather than allergic.

Atopy and milk protein IgE

A prior screening for atopy was reported by 36/46 (78%) of those with a positive food-screen. 26/36 (72%) had received a positive diagnosis either with skin prick tests or with a blood screen. Among those with a positive test result for milk-IgE antibodies, a previous diagnosis of allergy to animals or pollen was reported by 6/13 (46%). In the total study population, a previous diagnosis of atopy based on skin prick testing or RAST screening was reported by as many as 42% (349/857) of the subjects. The testing had been done either in childhood (n=129;37%), or at an adult age (n = 162;46%). 52 subjects (15%) had been tested positive for atopy, both in childhood and adulthood. 6 subjects (2%) did not report this information.

It is notable that IgE antibody levels for various food allergens reflect dietary habits (Schafer et al 2001; Jun et al 2006). Although dairy consumption in Finland is high (Järvelä 2005), the IgE levels for cow's milk protein in the present study were low compared to earlier studies (Sampson et al 2001; Sicherer et al 2006). Consumption of milk as a daily drink was not associated with a positive test result for IgE antibodies against cow's milk. A large number of those with a positive screen for major food allergens reported a previous, test-confirmed, positive diagnosis of atopy. Although the subjects were not tested for atopy, this implies a link between atopy and the presence of IgE antibodies for food antigens. It has been shown that atopic individuals are more prone to food hypersensitivity (Bjornsson et al 1996; Schafer et al 2001; Mattila et al 2003). A recent report from Finland shows that in school-aged atopic individuals, IgE antibodies to milk are common (Kolho et al 2005). Furthermore, the inhalant IgE antibody levels in the younger age cohorts have increased during recent years (Park et al 2006), so it was surprising that the number of positive results for IgE antibodies for food was not bigger among the youngest (18-33 years) than the oldest age group (50-64 years). However, the ethiopathogenesis of the presence of low IgE levels for food antigens in adults is obscure.

Main results

Low levels of food-specific IgE, for example for milk, do occur in adults, but a positive antibody level is seldom related to objective symptoms of hypersensitivity; hence measurement of milk protein specific IgE in primary care is not meaningful.

5.3.2 Milk protein IgA and IgG

In this study, a randomly-selected group of 400 subjects (from the original group of 1900) was evaluated. The evaluation showed that subjects drinking milk had higher levels of milk protein IgG in their sera than non-milk drinkers ($p < 0.001$), supporting the view that the presence of milk protein IgG specific antibodies may, to a certain degree, be a normal physiologic reaction to ingested milk protein. There was a positive correlation with milk-related GI-symptoms and milk protein IgG level: subjects reporting gastrointestinal problems after drinking milk had higher milk protein IgG levels, but consumed less milk than those who experienced no GI-symptoms. Dyspeptic subjects had lower milk protein IgG level than non-dyspeptics ($p < 0.05$). The association of high milk protein IgG level with constipation was close to the level of statistical significance. Diarrhoea had no association with milk protein IgG level ($p = 0.5$). Of minor symptoms, flatulence and bloating ($p = 0.8$), were not associated with milk protein IgG level. Milk protein IgA levels did not show any correlation to drinking milk. The frequency of specific GI-symptoms was not requested.

The levels of milk protein IgA and IgG declined as the age of the subjects increased, being lowest in the oldest age group; the age-related decline being statistically significant with milk protein IgG ($p < 0.004$). The age and personally estimated milk-related gastrointestinal problems had no correlation ($p = ns$). Men had higher milk protein IgA level in their sera than women ($p = 0.04$), but milk protein IgG level had no statistical significance gender-specifically.

Milk protein IgG or IgA and correlation with other inducers of GI-symptoms

Milk protein IgG was lower in subjects positive for antibodies to *Helicobacter pylori* (n=76/386, $p < 0.05$), although they drank milk more often than *Helicobacter pylori* negative subjects (n= 62/76, $p < 0.006$). However, the *Helicobacter pylori* positive group was somewhat older (mean age 46 years) than the *Helicobacter pylori* negative group (mean age 40 years, $p = 0.004$), which may explain the result. Furthermore, only 24/ 129 dyspeptic subjects were positive for *Helicobacter pylori* antibodies, showing that the negative correlation of IgG level with dyspepsia and *Helicobacter pylori* positivity are independent results. Accordingly, the presence of *Helicobacter pylori* antibodies in serum was associated statistically significantly to a lower level of milk protein IgA antibodies ($p = 0.03$)

There was no correlation between milk proteins IgG or IgA, and C/T-13910 genotype associated with adult type hypolactasia. Surprisingly, none of these subjects was screen-positive for coeliac disease (Study No. II). There was no correlation with milk specific IgG or IgA to a reported history of a diagnosed gastrointestinal disorder (irritable bowel syndrome n = 12/388 (3.0%) or inflammatory bowel disease n = 4/388 (1.0%)), since none of those patients had high levels of cow's milk specific IgG or IgA. Irritable bowel syndrome was reported less in the study group than in an average Western population (5-10%), and inflammatory bowel disease more often than in an average Western population (0.1%) (Colombel et al 2007; Hillilä et al 2007).

Milk protein IgG or IgA and clinical usefulness

There are studies showing that IgG4, which is a subgroup of IgG, might be useful in ruling out food intolerance due to its high negative predictive value (Bernardi et al 2008). Yet several others have shown no clear correlation between food intolerance and food specific IgG. Skripak et al showed that by sensitising cow's milk allergic children orally with milk there was an increase in milk specific IgG, especially in milk IgG4, but the milk specific IgE level did not change significantly (Skripak et al 2008). A positive correlation of a high level of food specific IgG4 in infancy and tolerance to corresponding food later in life has been shown (Tomicic et al 2009). A recent study from India (Poddar et al 2008) showed no correlation of cow's milk protein intolerance and the IgG anti-lactoglobulin antibody test, and recommended not using it in diagnosing cow's milk protein intolerance. Sletten et al showed a decrease in casein-specific IgE, IgG1 and IgG4 both in IgE-mediated and non-IgE-mediated CMA patients, whereas casein-specific IgA remains unchanged (Sletten et al 2007). A Dutch study showed that maintenance of tolerance in atopic children and adults to cow's milk in atopic children and adults without CMA was associated with elevated levels of milk-specific IgG4 in combination with low specific IgE (Ruiter et al 2007). There is also a recent study from Italy (Volpi et al 2009) showing that IgG could be used as a useful indicator of adverse reactions to food and food hypersensitivity. The clinical usefulness of the milk-specific IgG and its subgroups is still controversial, and even more the milk-specific IgA. In this study it was not possible to measure milk protein specific IgG4.

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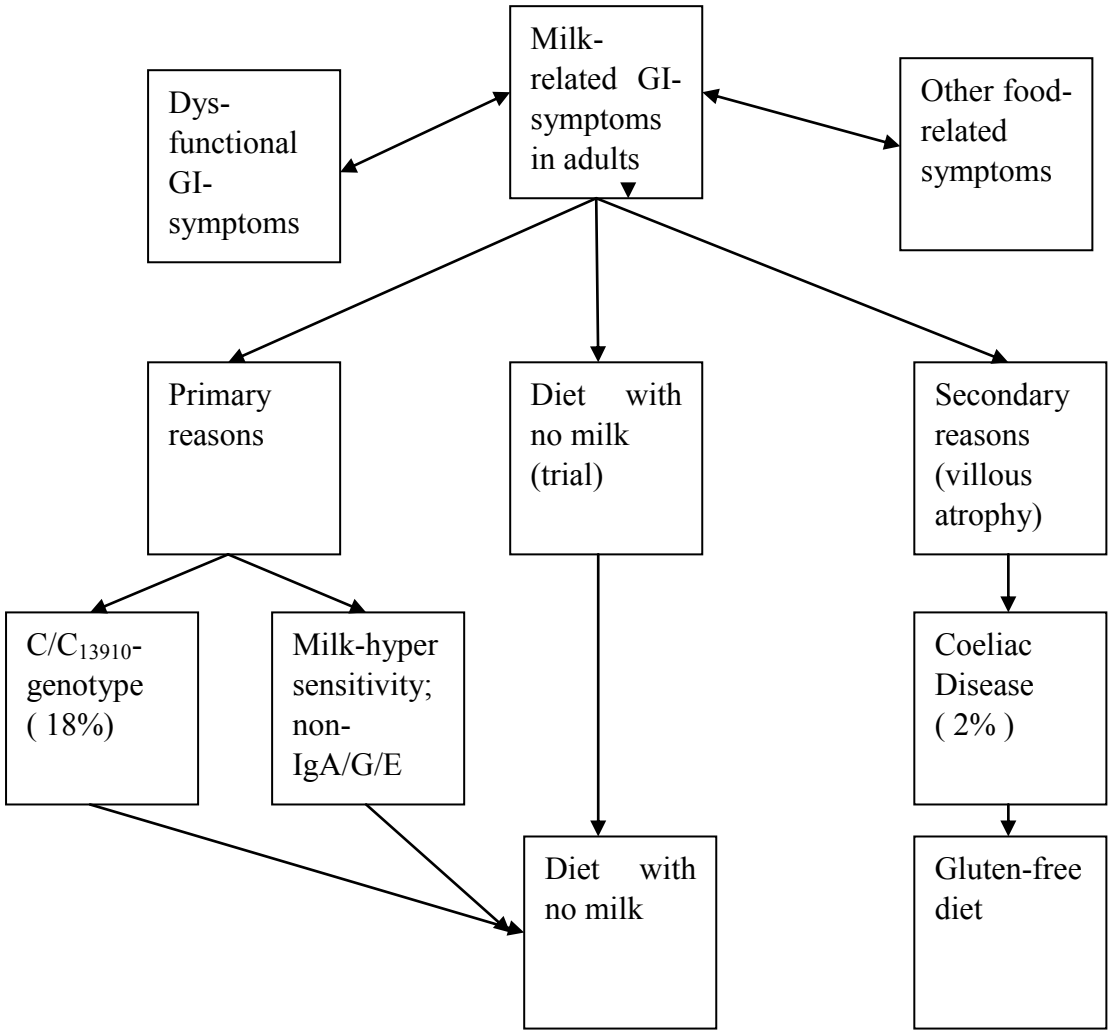
Main results

Milk protein IgG correlates with self reported milk-related GI-symptoms, whereas milk protein IgA has no such correlation. Milk protein IgG also correlates with drinking milk. This implies that milk protein specific IgG has a role in humoral reaction to ingested milk. However the measurement of milk protein IgG provides no accurate information on milk hypersensitivity.

5.3.3 How to approach GI-symptoms related to milk in adults in primary care

The approach to milk related symptoms in an outpatient clinic is best done systematically by eliminating possible primary and secondary reasons with screening, and by setting a milk-free trial period.

Figure 4 *The approach to milk-related problems*



6 Conclusion and future prospects

This study was large, and the goal to evaluate adult type food and especially milk- related symptoms and their correlation to specific screening methods in an outpatient setting was challenging. The seasonal variation was eliminated as we managed to collect all samples during a three-month period. Furthermore, the 99% response rate to the questionnaire was exceptionally high. This shows that people are interested in taking part in studies concerning their well-being, and even more when they receive valuable information which might help in resolving their possible health problems. Gastrointestinal symptoms are a common cause of diminished well-being and hence the participation rate of this study was excellent. Nevertheless, there are some confounding factors in this study which can bias the results: self-reported answers can have an effect on the results, since people's recollections are often not accurate, and the questions might be interpreted in various ways. However, the questionnaire was pretested on a small group of adults and its legibility and clearness were adjusted as well as possible. Since the participants were working-age persons, capable of understanding the questions, we can trust the answers to be accurate. Furthermore, the majority of participants were women, which can also somewhat bias the results. Since the prevalence of adult type hypolactasia was similar in this study group to previous studies in the Finnish population, it may be concluded that the study group represents the average Finnish population well.

Firstly, the question was set whether a gene-based test for adult type hypolactasia is applicable in clinical practice, and whether it should be used when diagnosing food and especially milk-related symptoms. And with no doubt the answer is yes. This test easily provides valuable and reliable information with a lifelong validity, and correlates well with the symptoms caused by adult type hypolactasia. The lactose tolerance test should no longer be used as a screening method of primary hypolactasia, due to its poor sensitivity and specificity as well as its relatively high cost and time consumption.

The second question was whether coeliac disease should actively be screened with new, easily accessible, and reliable non-invasive tests. The answer to this is also positive. The prevalence of undiagnosed coeliac disease is higher than previously thought. The symptoms of coeliac disease can be various. Besides gastrointestinal symptoms, there are numerous reports of different types of problems related to coeliac disease. Since the measurement of EmA or TG2A in association with IgA is easy and reasonably priced, this method should be used in outpatient clinics. However, a positive test result should be verified with gastroscopy and jejunal biopsy in accordance with current good clinical practice. The gluten-free diet is currently recommended if there are changes typical for coeliac disease in small bowel mucosa. It is yet to be seen whether future nutritional guidance might rely on non-invasive methods only.

The answer to the third question whether milk protein specific antibodies of group IgA, IgG or IgE should be measured among adults in diagnosing milk hypersensitivity in adults, is no. Their benefit to clinical judgement and diagnosing is, according to current knowledge, negative. Measurement of IgE with RAST-method is justified in atopy, even though prick testing gives more accurate results in most cases. In food related gastrointestinal symptoms, measurement of food specific IgE seldom gives further

information since low levels of food specific IgE are relatively common, and they do not correlate to the symptoms. Milk specific IgG is most likely a physiological reaction, although milk protein IgG level showed a positive correlation to self-reported milk related gastrointestinal problems in this study. Milk protein IgA does not seem to correlate with possible milk related problems. Furthermore, both milk protein IgG and IgA levels decreased towards older age groups in the study, the meaning of which is unclear.

The roles of milk protein IgA and IgG subgroups have also been studied, but the findings do not seem to give any further information; however, the milk protein IgG4 in milk hypersensitivity might have a role in the future. Milk protein IgE does not play an active role in milk induced gastrointestinal hypersensitivity in adults. Current belief is that a not yet characterized immunological type process might be behind the symptoms. The symptoms of food hypersensitivity are expressed individually also due to variation of bacterial flora in the colon, and due to individual sensitivity to feel distension in the colon as previously described. Also other food substances consumed (e.g. rye bread) might simultaneously be a reason for the symptoms.

The mechanism by which milk hypersensitivity in adulthood is mediated is yet to be answered.

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